OSMOTICALLY-INDUCED MODIFICATION OF ORNITHINE DECARBOXYLASE IN *PHYSARUM*

John L. A. MITCHELL and Grace E. KOTTAS

Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115, USA

Received 23 March 1979

1. Introduction

The activity of ornithine decarboxylase (ODC, EC 4.1.1.17) in tissues has been found to respond rapidly to variations in many growth parameters, including the ionic content of the media. Mammalian ODC activity is stimulated by hypotonic conditions [1], and inhibited by specific cations [2] or increased media osmolality [3], which is thought to be consistent with the enzyme's rate-limiting role in the biosynthesis of the polyamines. However, the mechanism of the critical correlation between ODC activity and variations in media ionic content has not been discovered. To this end we investigated this response in the lower eukaryote *Physarum polycephalum* where variations in ODC activity have been shown to be correlated with a reversible post-translational modification of this enzyme which markedly alters its affinity for the coenzyme, pyridoxal 5′-phosphate (PLP) [4,5].

As shown here, *Physarum* ODC was found to be unusually sensitive to minor changes in the osmolality of its media and, unlike previous reports, this response was extremely rapid and transitory, peaking in ~5 min and then reverting to control levels in <1 h. Furthermore this response was found to be entirely due to the post-translational modification of this enzyme, which resulted in the activation of as little as 18%, and up to 100%, of the available ODC enzyme. Thus osmotic shock may be used advantageously to investigate the mechanism and regulation of this enzyme form interconversion.

2. Experimental

2.1. *Culture techniques*

Exponentially-growing shake flask cultures of asynchronous microplasmodia of *Physarum polycephalum* were maintained as in [6].

2.2. *Sample preparations*

Microplasmodia were removed from their culture media by centrifugation at 200 × *g* for 30 s and immediately resuspended in an equivalent volume of the solution being tested and returned to the shaking platform. Samples were extracted at the appropriate time intervals and quickly placed in iced tubes centrifuged for 5 s at 250 × *g* in 4°C, decanted, and frozen in liquid nitrogen (total elapsed time of 40 s).

2.3. *Enzyme assay*

Frozen tissue pellets were sonified for 30 s in 10-times their volume of ice-cold 0.01 M EPPS (4-[2-hydroxyethyl]-1-piperazine propane sulfonic acid) buffer (pH 8.0) containing 0.5 mM dithiothreitol, 0.5 mM EDTA and 2 μM PLP. Duplicate 0.05 ml samples of this homogenate were placed in 25 ml Erlenmeyer flasks containing 1.9 ml 0.01 M EPPS buffer (pH 8.0) with 0.5 mM dithiothreitol, 0.5 mM EDTA, 0.5 μM PLP and 0.1 mM L-[1-^{14}C]ornithine (0.02 μCi). The flasks were sealed with rubber stoppers suspending center wells containing 0.1 ml hyamine hydroxide, and shaken at 26°C. After 1 h the reaction was stopped by injecting 1 ml 2 M citric acid and shaking continued for 30 min. Center wells were then

removed and counted in toluene based scintillation fluid at ~90% efficiency. This activity was attributed to the active form of the enzyme, ODC-A. Identical 0.05 ml homogenate samples were also assayed as above but in pH 8.4 EPPS buffer using 100 μM PLP. These conditions optimized the total assayable ODC activity. The difference between the activities determined by these two assays was attributed to the less active ODC-B [5].

2.4. *Osmolality determinations*

The osmotic activity of all solutions was determined by a vapor pressure osmometer.

3. Results

The ODC of actively growing *Physarum* microplasmodia generally is distributed almost equally between the active (ODC-A) and the less active (ODC-B) forms. Sudden alterations in the concentration of the standard culture media in the range from 0–250 $mOs.kg^{-1}$, did not induce any immediate variation in total ODC activity. As shown in fig.1, however, the proportion of this total activity attributed to each enzyme form was radically altered by relatively minor deviations from the standard 160 $mOs.kg^{-1}$ media. Reductions in the media osmolality stimulated an immediate increase in the A-form fraction to a maximum of 80–100% of total ODC within 10 min, followed by a return to approximately initial ratios within 60 min. Conversely, increased media osmolality induced the temporary conversion of ODC-A to the less active ODC-B with a peak response of ~20% ODC-A and ~80% ODC-B.

The combined results of many such media-induced alterations in ODC activity (fig.2) demonstrate that this temporary modulation in ODC activity correlates closely with media osmolality. Since similar responses obtained when NaCl, KCl, dextrose or diluted growth media were used to produce the gradations of media osmolalities, this response does not appear to be ion specific. The S-shaped curve of fig.2 also demonstrates that the greatest sensitivity occurred in the immediate vicinity of the osmotic concentration of the media in which the cells had been grown.

Osmotically-induced enzyme activation (increase in ODC-A) was completely blocked by simultaneous addition of sodium azide, or by decreasing the temperature of the media to 4°C (table 1). Conversely, osmotically-induced enzyme inactivation (decrease in ODC-A) was not affected by sodium azide, and was only partially inhibited by the lowering of the temperature to 4°C. The ODC of the cells resuspended in normal osmotic strength media, which maintained the initial form ratios at 26°C, was somewhat inactivated by the 4°C treatment.

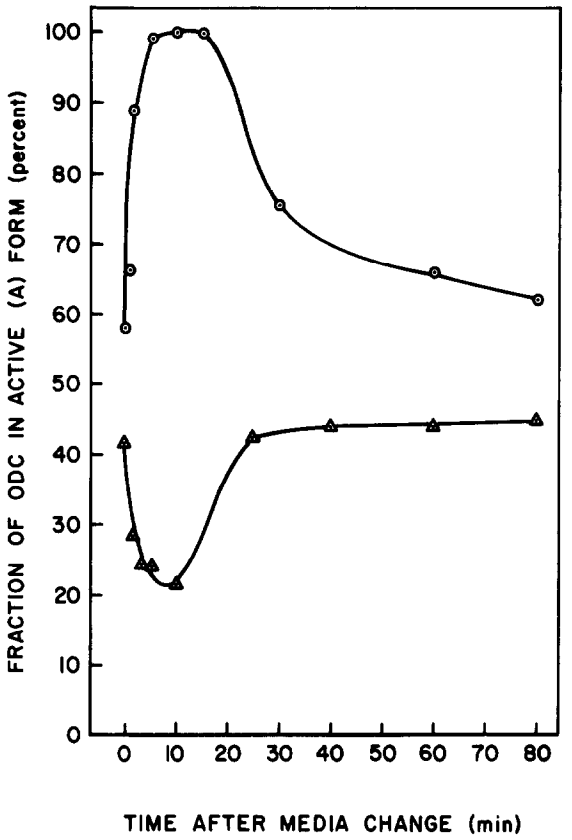

Fig.1. Time course of the variations in ODC following alteration of media osmolality. Log-phase microplasmodia cultured in 160 $mOs.kg^{-1}$ growth media were gently pelleted and resuspended in distilled water made to either 80 $mOs.kg^{-1}$ (○) or 200 $mOs.kg^{-1}$ (△) with NaCl. Samples extracted at the indicated intervals were assayed for A-form and total ODC activity, and results were expressed as the fraction of the total assayable ODC found as ODC-A. Control cultures resuspended in 160 $mOs.kg^{-1}$ (not shown) did not vary during this time period.

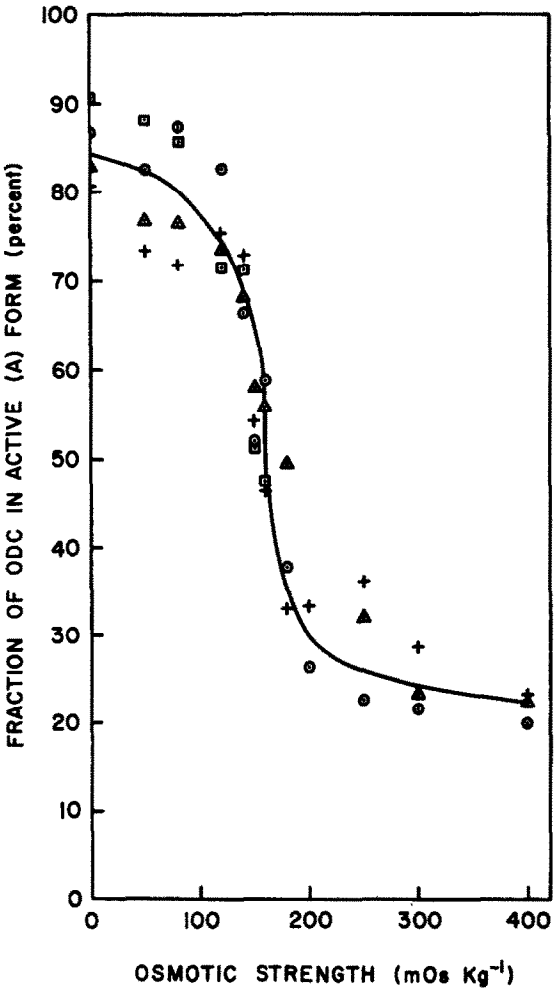

Fig.2. Correlation between osmotic strength and the temporary alteration in ODC activity. Log-phase microplasmodia were resuspended in either media diluted to the indicated osmolality (⊡), or distilled water adjusted to the indicated osmotic strength with KCl (⊙), NaCl (+) or dextrose (▲). Samples were harvested 6 min after suspension in these solutions and assayed as in fig.1.

Table 1
Effect of low temperature and sodium azide on the osmotically-induced response in ODC

Treatment	Conditions of treatment	Change in ODC-A (%)
Control	26°C	+3.2
(culture media)	26°C + sodium azide	+0.4
	4°C	−12.2
Activation	26°C	+85.8
(distilled water)	26°C + sodium azide	−19.0
	4°C	−16.3
Inactivation	26°C	−45.1
(220 mOs.kg^{-1} NaCl)	26°C + sodium azide	−53.2
	4°C	−20.5

Microplasmodia were resuspended in distilled water, 220 mOs. kg^{-1} NaCl or normal growth media as in fig.2 to induce the conversion of B enzyme to A, A to B, or serve as control (no change), respectively. Identical osmotic variations were also performed at 4°C or in the presence of 0.02% sodium azide. The amount of stimulation or inhibition observed in 6 min is presented as the % increase or decrease in ODC-A relative to its initial level. The data are the average values of 3 repeats of the entire experiments

4. Discussion

Although a correlation has been shown between media osmolality and ODC activity in eukaryotic tissues, the effect demonstrated here differs markedly in both speed and amplitude of this response. In mouse thyroid, for example, decreasing osmotic potential induced a very large increase in ODC activity which peaked after ~4 h [1]. While HeLa cells responded to decreased NaCl concentrations in about the same manner, their ODC activity was also shown to respond to sudden increases in NaCl concentration by quickly falling to undetectable levels (half-life of ~12 min) for ⩽24 h [3]. In all cases, the amplitudes of these responses were much greater than the 2–4-fold variations observed in *Physarum* ODC activity, and the times required for maximal response and return to steady state levels were in terms of hours instead of 4 and 60 min, respectively.

What is the significance of this osmotically-induced response in *Physarum*? Certainly the short time periods and relatively minor fluctuations reported here are not sufficient to produce major adjustments in polyamine levels, so it is unlikely that this response is an attempt to maintain osmotic balance through variation in polyamine concentration. Perhaps it is more reasonable to consider that the mechanism controlling ODC activity is sensitive to some temporary effect of osmotic shock such as the alteration of ion transport, net water flow, or disruption of membrane ionic potential due to the perturbation in membrane structure. In *E. coli*, for example, osmotic shock induces the loss of most cellular putrescine, and uptake of K^+

from the media, within 5 min [7]. A similar release of cellular polyamines to the media might be sufficient to induce inactivation of ODC in *Physarum* [8], yet this is not likely as the response time to extracellular polyamines has been shown to be considerably longer than the 4–6 min required for the osmotic effect. Furthermore the reabsorption of the released polyamine would not be fast enough to produce the observed rapid return to initial enzyme activity levels. It is more likely that exogenous polyamines [8] and osmotic shock, as well as the presence of inhibitors [9] and variations in growth and development [4,10], are all separate factors capable of influencing the common mechanism responsible for post-translational activation and inactivation of ODC.

The data presented here, as well as past studies on *Physarum* [8] and mammalian cells [1,2,11] correlating cell membrane integrity with intracellular ODC activity, suggest that the mechanism interconverting ODC forms may be located at the cell membrane. Furthermore, the inhibition of this osmotically-induced activation, but not inactivation, by sodium azide suggests that only the former reaction may be ATP dependent. However, both of these conclusions must be tempered by our current inability to clearly distinguish between affects on the activation–inactivation reactions per se, and those produced by altering the cell's perception of these enzyme-modulating signals. Clarification of the mechanism of regulation of this critical metabolic pathway will certainly be facilitated by this ability to easily induce the post-translational modification of ODC in vivo, by minor variations in media osmolality.

Acknowledgement

This study was supported by NIH Grant AM 17949.

References

[1] Friedman, Y., Park, S., Levasseur, S. and Burke, G. (1977) Biochem. Biophys. Res. Commun. 77, 57–64.
[2] Chen, K., Heller, J. S. and Canellakis, E. S. (1976) Biochem. Biophys. Res. Commun. 70, 212–220.
[3] Munro, G. F., Miller, R. A., Bell, C. A. and Verderber, E. L. (1975) Biochim. Biophys. Acta 411, 263–281.
[4] Mitchell, J. L. A., Anderson, S. N., Carter, D. D., Sedory, M., Scott, J. F. and Varland, D. A. (1978) Advances in Polyamine Research, vol. 1, pp. 39–50, Raven Press, New York.
[5] Mitchell, J. L. A. and Carter, D. D. (1977) Biochim. Biophys. Acta 483, 425–434.
[6] Mitchell, J. L. A. and Rusch, H. P. (1973) Biochim. Biophys. Acta 297, 503–516.
[7] Munro, G. F., Hercules, K., Morgan, J. and Sauerbier, W. (1972) J. Biol. Chem. 247, 1272–1280.
[8] Mitchell, J. L. A., Carter, D. D. and Rybski, J. A. (1978) Eur. J. Biochem. 92, 325–331.
[9] Mitchell, J. L. A., Campbell, H. A. and Carter, D. D. (1976) FEBS Lett. 62, 33–37.
[10] Sedory, M. J. and Mitchell, J. L. A. (1977) Exp. Cell Res. 107, 105–110.
[11] Chen, K., Heller, J. and Canellakis, E. S. (1976) Biochem. Biophys. Res. Commun. 68, 401–408.